

Molecular Phylogenetic Relationships of *Cibotium* and Origin of the Hawaiian Endemics

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ABSTRACT.—The tree fern genus *Cibotium* comprises nine species distributed in tropical regions of Asia, Mesoamerica, and the Hawaiian Islands. The four Hawaiian species are endemic to the Hawaiian Islands. The goals of this paper were to determine the relationships among the *Cibotium* species, determine whether the Hawaiian species are monophyletic, and infer the dispersal pathway likely responsible for delivering an ancestral *Cibotium* species to the Hawaiian Islands. Molecular phylogenetic analyses based on four coding and five non-coding plastid DNA sequences supported Hawaiian *Cibotium* as monophyletic, suggesting a single colonization of the Hawaiian Islands. Hawaiian *Cibotium* are most closely related to species in Mesoamerica. If the ancestor of Hawaiian *Cibotium* dispersed to the Hawaiian Islands via wind dispersed spores, our analyses suggest the trade winds or storms delivered spores from Mesoamerica or the Hawaiian Islands were colonized first by a species from Asia, followed by subsequent dispersal to Mesoamerica from Hawai'i. Our analyses do not allow us to favor one hypothesis over the other.

KEY WORDS.—Hawai'i, historical biogeography, *Cibotium*, spore dispersal, molecular phylogenetics, tree ferns

The current high islands of the Hawaiian Islands are located approximately 4000 km from the nearest continent, North America, and 1600 km from the nearest archipelago, the Marquesas Islands of Polynesia. The Hawaiian Island chain is about 80 million years old and was produced in a conveyer-belt-like manner by the Hawaiian hotspot beneath the Pacific Ocean. Islands are removed from the hotspot as the Pacific tectonic plate moves to the northwest (e.g., Clague and Dalrymple, 1987). Once removed from the hotspot, the islands eventually erode to or below sea level. Geological evidence suggests that the islands' separation from the mainland has been relatively constant (Carson and Clague, 1995) and their extreme isolation has been a barrier to colonization by terrestrial organisms. Because the islands are volcanic in origin and are so isolated, colonizing species must have dispersed to the islands via wind or water.

The Hawaiian Islands have the highest levels of species endemism of any regional flora in the world (Wagner *et al.*, 1999), which can be partially explained by the continual opening of new habitats accompanied by dispersal of organisms throughout the developing islands. Of the 136 species of ferns and lycophytes native to the Hawaiian Islands, 67% are endemic to the archipelago (Vernon, 2011). Ferns and lycophytes comprise about 12% of the native vascular-plant species of the Hawaiian Islands (Warren L. Wagner, 2011 pers. comm. and <http://botany.si.edu/pacificislandbiodiversity/hawaiianflora/>), although they constitute only about 3% of the vascular-plant flora of the world.

During the past 80 Ma the size of islands produced by the activity of the Pacific hotspot has varied (Price and Clague, 2002). Price and Clague (2002) indicate that there was a lull in island formation from 33 Ma to 23 Ma ago. During this time, the lack of available habitats would have resulted in extinction of most or all Hawaiian terrestrial biota such that there was essentially a renewal of terrestrial life beginning 23 Ma ago. Prior to 18 Ma ago, there were few islands with peaks over 1000 m, but between approximately 18 Ma and 11 Ma ago there were numerous larger peaks over 1000 m, including some greater than 2000 m. However, due to subsequent erosion of those larger peaks, the models produced by Price and Clague (2002) indicate there were few islands higher than 1000 m during the 5–6 Myr preceding the formation of Kauai 5.2 Ma ago. Because of this, Price and Clague (2002) suggested that montane taxa, including most of the ferns, probably arrived from outside the Hawaiian archipelago or evolved after the formation of Kauai approximately 5.2 Ma ago because appropriate mid- and high-elevation montane habitats did not exist on islands older than Kauai by the time Kauai was high enough to support these habitats.

Geiger *et al.* (2007; and references therein) identified four potential weather- or climate-based pathways for spore dispersal that could explain the biogeographic origins of native Hawaiian fern lineages. They suggested the northern subtropical jetstream could carry spores to the Hawaiian Islands from areas of the Indo-Pacific; spores could be moved from Central and North America via the trade winds; spores could be carried by storms from southern Mesoamerica; or spores could be transported from the Southern Hemisphere (S. America or the South Pacific) northward across the equator by a combination of mechanisms involving a seasonal southern shift of the Intertropical Convergence Zone (ITCZ), movement of air via Hadley Cells, and the trade winds (see Wright *et al.*, 2001). Reviews of studies by Geiger *et al.* (2007) that included Hawaiian endemic species (*Dryopteris* (Geiger and Ranker, 2005), *Hymenophyllum* (Ebihara *et al.*, 2004; Hennequin *et al.*, 2006), *Polystichum* (Driscoll and Barrington, 2007), *Adenophorus*, *Grammitis*, and *Stenogrammitis* (Ranker *et al.*, 2003, 2004; *Stenogrammitis* treated as *Lellingeria* in the works cited)) found evidence for each of the four described weather/climate-based dispersal mechanisms. In the current study, using molecular phylogenetics, we evaluated the biogeographical history of the

genus *Cibotium*, which includes four Hawaiian endemic species, in light of the four weather/climate-based dispersal mechanisms.

The monophyletic tree fern genus *Cibotium* (Cibotiaceae) (Korall *et al.*, 2006; Smith *et al.*, 2006) comprises nine species (see Table 1) (Kramer 1990c; Palmer, 1994). In the Hawaiian Islands it is represented by four endemic species, in Asia by three species, and in Mesoamerica by two species (Kramer, 1990c; Palmer, 1994). The genus is characterized by large rhizomes, leaves 2–4 m in length, hairy petiole bases, and a solenostele or dictyostele (Smith *et al.*, 2006). The spores of *Cibotium* show varying degrees of exine distal ridge development (Gastony, 1982). The spores of Asian species have the greatest degree of distal and equatorial ridge development, while those of the Hawaiian species intergrade with those from the Mesoamerican species, which have the least developed ridges (Gastony, 1982).

Fosberg (1948), who hypothesized geographic origins for the original colonizing ancestors of native Hawaiian ferns and lycophytes, hypothesized one colonizing ancestral species for Hawaiian *Cibotium* that probably originated in the Indo-Pacific, but he could not rule out the possibility of an American origin. The general goal of this research was to test Fosberg's (1948) hypothesis using molecular phylogenetic analyses, and to specifically: 1) determine if the Hawaiian species of *Cibotium* are monophyletic; and 2) determine the relationships among the nine species of *Cibotium*, elucidating the biogeographic origin(s) of the Hawaiian species, and thus identifying which climate-based dispersal pathway (Geiger *et al.*, 2007) most likely explains how the ancestor(s) of the Hawaiian species was delivered to the Hawaiian Islands.

MATERIALS AND METHODS

Sampling and molecular methods.—All nine species of *Cibotium* were sampled in this study, as well as three outgroup species chosen based on relationships revealed by Korall *et al.* (2006). The three outgroup taxa, *Calochlaena villosa*, *Dicksonia antarctica*, and *Lophosoria quadripinnata*, belong to the Dicksoniaceae as circumscribed by Smith *et al.* (2006), a well-supported group closely related to *Cibotium*. Representatives of the genus *Metaxya* were not included as outgroups due to their unclear phylogenetic position (Korall *et al.*, 2006). Taxa included in this study, voucher information, and database accession numbers are listed in Table 1.

Total DNA was extracted using the DNeasy plant mini kit from Qiagen (Valencia, CA, USA). Four coding (*atpA*, *atpB*, *rbcl*, *rps4*) and five non-coding (*rbcl-atpB*, *rbcl-accD*, *rps4-trnS*, *trnG-R*, and *trnL-F*) plastid DNA regions were PCR amplified and sequenced. When possible, sequences accessible through GenBank were utilized. In Geiger's lab, *rbcl* and *trnL-F* were amplified as in Geiger and Ranker (2005), amplification of *atpB* followed the methods described in Ranker *et al.* (2003), amplification of *trnG-R* followed the methods of Korall *et al.* (2007), and the *rps4-trnS* IGS was amplified following

TABLE 1. Species list, collection, and voucher information, and database accession numbers.

Taxa examined in study					
Species	Family	Voucher	Fern DNA DB#	<i>atpA</i>	<i>atpB</i>
Ingroup					
<i>Cibotium arachnoideum</i> (C.Chr.) Holttum	Cibotiaceae	Ranker 2010 (COLO; TAIF)	NA	JX485654 ³	JX485655 ³
<i>Cibotium barometz</i> (L.) J.Sm.	Cibotiaceae	Larsen et al. 46302 (AAU)	2277	AM176429 ¹	AM176589 ¹
<i>Cibotium chamissoi</i> Kaulf.	Cibotiaceae	Morter 2 (E)	2486	AM176430 ¹	AM176590 ¹
<i>Cibotium cumingii</i> Kunze	Cibotiaceae	Ranker 2070 (COLO; TAIF)	NA	NA	JX485656 ³
<i>Cibotium glaucum</i> (Sm.) Hook. & Arn.	Cibotiaceae	Wolf 266 (Strybing Herbarium)	132	AM176431 ¹	AM176591 ¹
<i>Cibotium menziesii</i> Hook.	Cibotiaceae	Ranker 1996 (COLO; BISH)	NA	NA	JX485657 ³
<i>Cibotium nealiae</i> Degen.	Cibotiaceae	Fagerlind & Skottsberg 6494 (S)	2253	AM176432 ¹	AM176592 ¹
<i>Cibotium regale</i> Versh. & Lem.	Cibotiaceae	Oxford Botanic Garden	NA	NA	JX485658 ³
<i>Cibotium regale</i> Versh. & Lem.	Cibotiaceae	Royal Botanic Garden 19991656	NA	NA	—
<i>Cibotium schiedei</i> Schlecht. & Cham.	Cibotiaceae	Morter 4 (E)	2485	AM176433 ¹	AM176593 ¹
Outgroup					
<i>Calochlaena villosa</i> (C. Chr.) M. D. Turner & R. A. White	Dicksoniaceae	Woodhaus (AAU)	2254	AM176428 ¹	AM176588 ¹
<i>Dicksonia antarctica</i> Labill.	Dicksoniaceae	Wolf 276 (UTC)	134	AM176442 ¹	U93829
<i>Lophosoria quadripinnata</i> (J. F. Gmel.) C. Chr. (Type sp.)	Dicksoniaceae	Grantham 006-92 (UC)	424	AM176450 ¹	AM176609 ¹

A dash (—) indicates that data are available for this taxon from a different voucher

XXX = new to this study, accession numbers inserted later

NA = Data not available for this taxon

¹ from Korall et al., 2006

² from Korall et al., 2007

³ sequenced by JMOG

⁴ sequenced by PK

Bold – from other studies

Li and Lu (2006). PCR products were purified with ExoSAP-IT (USD Corp.) and sequenced by Macrogen Inc. in South Korea. Sequences produced by Korall were amplified and sequenced using the primers and methods described in Korall et al. (2006, *atpA*, *atpB*, *rbcl*, *rps4*, and *rps4-trnS* IGS

TABLE 1. Extended.

<i>rbcL</i>	<i>rps4</i>	<i>rbcL-accD</i>	<i>rbcL-atpB</i>	<i>trnGR</i>	<i>trnLF</i>	<i>rps4-trnS</i> IGS
JX485690 ³	NA	NA	NA	NA	NA	NA
AM177328 ¹	AM176485 ¹	JX485694 ⁴	NA	JX485674 ⁴	JX485682 ⁴	JX485663 ⁴
AM177329 ¹	AM176486 ¹	JX485695 ⁴	JX485659 ⁴	JX485675 ⁴	JX485683 ⁴	JX485664 ⁴
JX485691 ³	NA	NA	NA	JX485676 ³	JX485684 ³	JX485665 ³
U05913	AM176487 ¹	JX485696 ⁴	JX485660 ⁴	JX485677 ⁴	JX485685 ⁴	JX485666 ⁴
JX485692 ³	NA	NA	NA	JX485678 ³	JX485686 ³	JX485667 ³
AM177330 ¹	AM176488 ¹	JX485697 ⁴	JX485661 ⁴	JX485679 ⁴	JX485687 ⁴	JX485668 ⁴
—	NA	NA	NA	JX485680 ³	JX485688 ³	JX485669 ³
JX485693 ³	NA	NA	NA	—	—	—
AM177331 ¹	AM176489 ¹	JX485698 ⁴	JX485662 ⁴	JX485681 ⁴	JX485689 ⁴	JX485670 ⁴
AM177327 ¹	AM176484 ¹	AM410497 ²	AM410290 ²	AM410426 ²	AM410354 ²	JX485672 ⁴
U05919	AF313596	AM410498 ²	AM410291 ²	AM410427 ²	AM410355 ²	JX485671 ⁴
AF101303	AM176502 ¹	AM410505 ²	AM410298 ²	AM410434 ²	AM410361 ²	JX485673 ⁴

(amplified and sequenced within *rps4*) and Korall *et al.* (2007, *rbcL-accD*, *rbcL-atpB*, *trnG-R*, and *trnL-F*).

Sequence editing and alignment.—Sequence fragments were assembled and edited using Sequencher version 4.2.2 (Gene Codes, Ann Arbor, Michigan, USA). The edited consensus sequences were aligned manually using MacClade version 4.07b13 (Maddison and Maddison, 2005). Ambiguously aligned regions (due to insertions or deletions) in the noncoding regions (*rbcL-accD*, *rbcL-atpB*, *trnG-R*, *trnL-F*, and *rps4-trnS* IGS) were excluded from the analyses. No “gap coding” was performed.

TABLE 2. Number of characters, summary of nucleotide substitution models used in Bayesian (B/MCMC) analysis, and sequence analysis for the nine ingroup and three outgroup taxa.

Data set	No. char	B/MCMC substitution model
<i>atpA</i>	1514	HKY+I
<i>atpB</i>	1482	HKY
<i>rbcL</i>	1309	HKY+G
<i>rbcL-accd</i>	1452	GTR+I
<i>rbcL-atpB</i>	640	HKY+G
<i>rps4</i>	603	HKY
<i>rps4-trnS</i>	466	HKY+G
IGS		
<i>trnGR</i>	1050	HKY+G
<i>trnLF</i>	1065	HKY+I
Combined		-----
Aligned	9581	
Excluded	105	
Analyzed	9476	

Phylogenetic analyses.—We tested for incongruence among the nine single-region data sets by analyzing the data sets separately using both a Bayesian Markov chain Monte Carlo approach (B/MCMC) and equally weighted Maximum Parsimony (MP) with settings as described below for the combined data set. We compared the resultant topologies and incongruence supported by a Bayesian posterior probability of $\geq 70\%$ was considered a conflict. For each analytical method we compared all nine resulting topologies and no conflicts were found in the ingroup. The nine single-region data sets were combined into a single data set and analyzed using a Bayesian Markov chain Monte Carlo approach (B/MCMC) and equally weighted Maximum Parsimony (MP). B/MCMC analyses were performed with MrBayes 3.1.1 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003), using a single partition for each region (i.e., with nine partitions). The most appropriate nucleotide substitution models for each of the regions were determined using the Perl script MrAIC version 1.4.4 (Nylander, 2004) in combination with PHYML version 2.4.4 (Guindon and Gascuel, 2003). The choice of model was based on the corrected Akaike information criterion (AICc) (see Table 2 for a summary of models used). The analysis was run for five million generations, on four parallel chains, with the temperature parameter set to 0.2. Four independent analyses of each region were run simultaneously. To determine whether parameters had converged, the values sampled for different parameters were examined using the program Tracer v. 1.2.1 (Rambaut and Drummond, 2005). We also examined the standard deviation of the split frequencies among the independent runs as calculated by MrBayes. For each analysis, every 1000th tree was sampled, and burn-in was conservatively set to one million generations. A majority-rule consensus tree was calculated based on the pool of trees resulting from the four independent analyses (except those trees discarded as burn-in).

The MP analyses were performed with PAUP* version 4.0b10 (Swofford, 2002) and included a heuristic search for the most parsimonious trees with 1000 random-sequence-addition replicates with MulTrees activated and tree-bisection-reconnection (TBR) branch swapping. Support for nodes was calculated by bootstrap analysis with 5000 bootstrap replicates with 10 random-sequence-addition replicates each.

All trees were rooted with the three outgroup species.

RESULTS

The number of characters included in the analyses and the nucleotide substitution model used in B/MCMC analyses are summarized in Table 2. For the combined MP analysis 407 (4.3%) characters were parsimony informative. The heuristic MP search resulted in three most parsimonious trees, each with a length of 977 steps in one island.

Our results indicated that, based on plastid data alone, *Cibotium* was strongly supported as monophyletic and most relationships among the *Cibotium* species were strongly supported (posterior probability, PP = 1.00) according to the B/MCMC analysis, and they were moderately (bootstrap percentage, BP = 70–89%) to strongly supported by the MP analysis (BP \geq 90%) (Fig. 1). Few indels were found, and these were either autapomorphies or synapomorphies for clades already well supported. Most indels supported the monophyly of the ingroup. Only two indels were synapomorphies of subclades within the ingroup: a five-base indel joining *Cibotium regale* and *C. schiedei* and a seven-base indel shared by *C. chamissoi* and *C. menziesii*.

Within *Cibotium*, there were three strongly supported clades that correspond to the geographic distributions of the included species: the Asian clade, with three species, was sister to a clade with the four Hawaiian species in one subclade, and the two Mesoamerican species in the other. Within the Asian group, *C. arachnoideum* was well supported by B/MCMC analysis and moderately supported by MP analysis as sister to the pair *C. barometz* and *C. cumingii*. The sister relationship between the Hawaiian species and those from Mesoamerica was strongly supported by both analyses. Within the Hawaiian clade, *C. chamissoi* and *C. menziesii* were strongly and moderately supported as sister species by B/MCMC and MP analyses, respectively. However, the relationships among those two taxa and *C. nealiae* and *C. glaucum* were unresolved.

DISCUSSION

The goals of this study were to determine if the Hawaiian species of *Cibotium* are monophyletic and to determine the relationships among species of *Cibotium* to infer the biogeographical history of the Hawaiian species. Using five coding and four non-coding plastid DNA loci, this study has resulted in a relatively well-resolved molecular phylogeny for *Cibotium*. Within *Cibotium*, three clades were resolved, each of which corresponds to the geographical

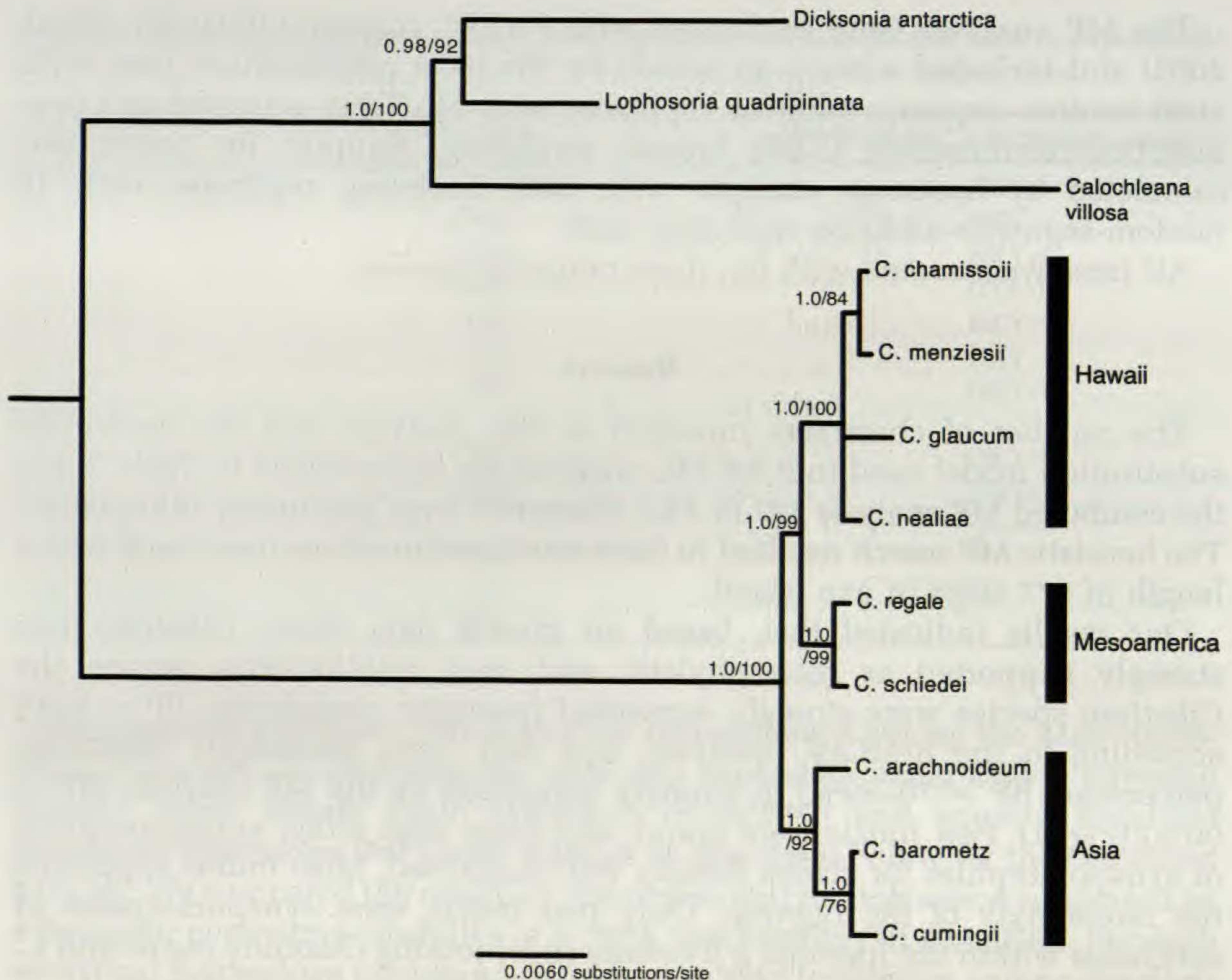


FIG. 1. The 50% majority-rule consensus tree resulting from Bayesian (B/MCMC) analyses of the combined data set (four coding (*atpA*, *atpB*, *rbcL*, *rps4*) and five non-coding (*rbcL-atpB*, *rbcL-accD*, *rps4-trnS* IGS, *trn-GR*, and *trnL-F*). Support values from Bayesian analysis (posterior probabilities (PP)) are in front of the slash, and support values from maximum parsimony (MP) are behind the slash.

distributions of the component species. We do interpret the results with caution, however, as the phylogeny is solely based on plastid data.

The four endemic Hawaiian *Cibotium* species comprise a well-supported clade (Fig. 1). Based on this, we infer there was a single *Cibotium* ancestral species that colonized the Hawaiian Islands, followed by subsequent diversification of the species. The Hawaiian clade is strongly supported as sister to *C. regale* and *C. schiedei*, which have Mesoamerican distributions. The Asian clade is strongly supported as sister to the Hawaiian + Mesoamerican clades.

The sister relationship between the Hawaiian and Mesoamerican clades is supported by spore morphology as described by Gastony (1982). His studies indicated that the spores of Asian species have the greatest degree of exine ridge development, while those of the Hawaiian and Mesoamerican species show an intergradation, with the Mesoamerican species having the least developed ridges.

Based on the phylogeny obtained and the well-supported relationship between the Hawaiian and Mesoamerican clades, there are three possible biogeographic histories. First, it is possible that the ancestral species dispersed to the Hawaiian Islands via the trade winds or storms from Mesoamerica as described by Geiger *et al.* (2007). Second, it is possible that the Hawaiian Islands were colonized before Mesoamerica by an Asian species reaching the Hawaiian Islands via the jetstream. Subsequently, there could have been dispersal of the established Hawaiian species to Mesoamerica. Third, there could have been two independent dispersal events of the same ancestral species out of Asia, one to the Hawaiian Islands and the other to Mesoamerica. This scenario seems much less likely than either of the other two.

Geiger *et al.* (2007) reviewed molecular phylogenies and biogeographical histories of six genera that are represented in the Hawaiian Islands by endemic species. For each of the endemic lineages, they analyzed molecular phylogenies to develop hypotheses about the potential climate/weather-based pathway utilized to disperse to the Hawaiian Islands. They hypothesized that the northern subtropical jetstream was the pathway responsible for delivering ancestral spores of three of five endemic lineages of *Dryopteris* (Geiger and Ranker, 2005), the two endemic *Polystichum* lineages (Driscoll and Barrington, 2007), potentially one lineage of *Hymenophyllum* (Ebihara *et al.*, 2004; Hennequin *et al.*, 2006), and maybe one lineage of *Grammitis* (Ranker *et al.*, 2004; Geiger *et al.*, 2007) to the Hawaiian Islands. The trade winds or storms were the implicated pathways responsible for delivery of *Adenophorus* (Ranker *et al.*, 2003) and possibly of *Stenogrammitis* to the Hawaiian Islands from the Neotropics. The Hadley Cell shift mechanism (Wright *et al.*, 2001) could not be eliminated as a possibility for either *Grammitis* or *Stenogrammitis*.

As stated above, we are unable to eliminate either Asia or Mesoamerica as the source area of the *Cibotium* spores that colonized the Hawaiian Islands based on the topology presented here. Our results suggest it is possible that spores were delivered from Asia via the northern subtropical jetstream or from Mesoamerica via storms or the trade winds to the Hawaiian Islands. Even though the large amount of molecular data used has allowed us to produce a well-supported topology, the relationships resolved in this study do not allow us to favor one dispersal hypothesis over the other. However, a recent biogeographical study of scaly tree ferns, Cyatheaceae, by Korall and Pryer (unpublished data) estimated clade divergence times, and included one Asian, one Mesoamerican, and one Hawaiian *Cibotium* species in the analyses. The dating results suggest that the crown group of *Cibotium* originated 24–25 Ma ago, and the Mesoamerican and Hawaiian clades separated from each other about 17–18 Ma ago. Since Price and Clague (2002) estimated that appropriate Hawaiian montane habitats would not have been present prior to about 5.2 Ma ago when Kauai formed, the hypothesized dispersal route of Asia to the Hawaiian Islands to Mesoamerica via the jetstream to explain the sister relationship of the Hawaiian and Mesoamerican clades would seem unlikely and a biogeographical explanation of dispersal from Asia to Mesoamerica to the Hawaiian Islands via the trade winds or a storm should be favored.

TABLE 3. Geographic distribution and elevation ranges for extant *Cibotium* species.

Species	Native distribution	Elevation range	Reference
<i>Cibotium arachnoideum</i>	SE Asia	Lower montane forest in East Malaysia, 700–2100 m on Mt. Kinabalu [Borneo]	Large and Braggins, 2004
<i>Cibotium barometz</i>	SE Asia	200–1700 m	Large and Braggins, 2004
<i>Cibotium chamissoi</i>	Hawai'i – all major islands except Kaua'i	150–1200 m, as low as 50 m, less common above 800 m	Palmer, 2003
<i>Cibotium cumingii</i>	SE Asia	no information	no information
<i>Cibotium glaucum</i>	Hawai'i – all major islands	300–1700 m, as low as 100 m; is the most common <i>Cibotium</i> above 800 m	Palmer, 2003
<i>Cibotium menziesii</i>	Hawai'i – all major islands	250–1400 m	Palmer, 2003
<i>Cibotium nealiae</i>	Hawai'i – Kaua'i only	135–1300 m	Palmer, 2003
<i>Cibotium regale</i>	Mesoamerica	700–2900 m 1140–2200 m	Davidse <i>et al.</i> , 1995 Mickel and Smith, 2004
<i>Cibotium schiedeii</i>	Mesoamerica	600–1400 m	Mickel and Smith, 2004

Although the above argument would support a Mesoamerican origin for Hawaiian *Cibotium*, we do not feel we can favor that hypothesis over an Asian origin hypothesis for two reasons. First, although extant species of *Cibotium* seem to favor somewhat higher elevations (500–2500 m), for example in submontane forests (Large and Braggins, 2004), all of the Hawaiian species are reported to occur at 250 m or lower, and *C. chamissoi* is known to occur as low as 50 m (Table 3), thus early colonists (at least based on elevation) theoretically could have survived on the lower elevation islands that existed before the formation of Kaua'i. Much like what was shown for “diellia”, a Hawaiian endemic clade of *Asplenium* (Schneider *et al.*, 2005), *Cibotium* could have been one of the early colonizers of Kaua'i having dispersed from an adjacent, lower elevation island. Based on the estimated 17–18 Ma ago split between Mesoamerican and Hawaiian clades, it is possible an ancestral Hawaiian species could have also dispersed to Mesoamerica during that time period. In support of this hypothesis, Hutsemékers *et al.* (2011) have shown that at least some spore-producing plants do not lose their ability to disperse as seed plants often do when they become established on oceanic islands. Second, Vernon and Ranker (2013) have estimated that for the fern and lycophyte taxa that have been studied, the largest proportion of Hawaiian taxa have their closest relatives in the old world, presumably due to the greater efficiency of the jetstream in delivering spores to the Hawaiian Islands compared to the possible mechanisms from other regions. Sixty-one percent of native Hawaiian

taxa were estimated to have their closest relatives in Asia/Paleotropics, whereas only 13% have their closest relatives in the Americas/Neotropics.

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